

This listing of claims will replace all prior versions, and listing, of claims in the application:

In the Claims

Claim 1 (original): A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) hybridizing a polynucleotide comprising a termination polynucleotide sequence to a DNA template-composite primer complex, wherein said complex comprises a composite primer hybridized to a single stranded DNA template comprising the target sequence, said composite primer comprising an RNA portion and a 3' DNA portion,

whereby said polynucleotide comprising a termination polynucleotide sequence is hybridized to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(b) extending the composite primer in the DNA template-composite primer complex of step (a) with DNA polymerase;

(c) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

Claim 2 (original): A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) extending a composite primer in a complex comprising (i) a single stranded DNA template comprising the target sequence; and (ii) the composite primer, said composite primer comprising an RNA portion and a 3' DNA portion, wherein the DNA template is hybridized to the composite primer;

(b) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

Claim 3 (original): A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

(a) extending a composite primer in a complex comprising (i) a single stranded DNA template comprising the target sequence; (ii) the composite primer, said composite primer comprising an RNA portion and a 3' DNA portion, wherein the composite primer is hybridized to the DNA template; and (iii) a polynucleotide comprising a termination polynucleotide sequence, wherein the polynucleotide comprising a termination polynucleotide sequence is hybridized to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(b) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

Claim 4 (original): A method for amplifying a polynucleotide sequence complementary to a target polynucleotide sequence comprising:

cleaving an RNA portion of a composite primer extension product in a complex comprising:

(a) a single stranded DNA template comprising the target sequence; and

(b) the composite primer extension product, wherein the composite primer of the composite primer extension product comprises an RNA portion and a 3' DNA portion, wherein the composite primer extension product is hybridized to the DNA template;

wherein said cleaving is with an enzyme that cleaves RNA from an RNA/DNA hybrid, whereby another composite primer hybridizes to the target polynucleotide sequence and repeats primer extension by strand displacement,

whereby multiple copies of the complementary sequence of the target sequence are produced.

Claim 5 (original): A method for amplifying a target polynucleotide sequence comprising generating displaced primer extension product using the method of any of claims 1-4, further comprising:

hybridizing a polynucleotide comprising a propromoter and a region which hybridizes to the displaced primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the displaced primer extension products,

whereby multiple copies of the target sequence are produced.

Claim 6 (original): A method for amplifying a target polynucleotide sequence comprising:

hybridizing a primer extension product with a polynucleotide comprising a propromoter and a region which hybridizes to the primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the primer extension product, wherein the primer extension product is a displaced primer extension product generated by:

(a) hybridizing a single stranded DNA template comprising the target sequence with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(c) extending the composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement to produce displaced primer extension product; whereby multiple copies of the target sequence are produced.

Claim 7 (original): A method for amplifying a target polynucleotide sequence comprising:

(a) hybridizing a first composite primer to a single stranded DNA template comprising the complementary sequence of the target polynucleotide sequence, said composite primer comprising an RNA portion and a 3' DNA portion, wherein said single stranded DNA template is generated by a method comprising:

(i) hybridizing a polynucleotide comprising the target polynucleotide sequence with a second composite primer, said second composite primer comprising an RNA portion and a 3' DNA portion;

(ii) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the polynucleotide comprising the target polynucleotide sequence which is 5' with respect to hybridization of the second composite primer to said polynucleotide;

(iii) extending the second composite primer with DNA polymerase;

(iv) cleaving the RNA portion of the annealed second composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the polynucleotide comprising the target polynucleotide sequence and repeats primer extension by strand displacement, whereby multiple copies of a single stranded DNA template comprising the complementary sequence of the target polynucleotide sequence are generated;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the first composite primer to the template;

(c) extending the first composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed first composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement,
whereby multiple copies of the target polynucleotide sequence are produced.

Claim 8 (original): The method of any of claims 1-4 and 6, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

Claim 9 (original): The method of claim 5, wherein the RNA portion of the composite primer is 5' with respect to the 3' DNA portion.

Claim 10 (original): The method of claim 7, wherein the RNA portion of the first composite primer and the second composite primer is 5' with respect to the 3' DNA portion.

Claim 11 (original): The method of claim 8, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

Claim 12 (original): The method of claim 9, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

Claim 13 (original): The method of claim 10, wherein the 5' RNA portion is adjacent to the 3' DNA portion.

Claims 14-21 (canceled)

Claim 22 (original): The method of any of claims 1-4 and 6, wherein the enzyme that cleaves RNA is RNaseH.

Claim 23 (original): The method of claim 5, wherein the enzyme that cleaves RNA is RNaseH.

Claim 24 (original): The method of claim 7, wherein the enzyme that cleaves RNA is RNaseH.

Claims 25-47 (canceled)

Claim 48 (original): A method for amplifying a target polynucleotide sequence comprising:

(a) hybridizing a single stranded DNA template comprising the target sequence with a composite primer, said composite primer comprising an RNA portion and a 3' DNA portion;

(b) optionally hybridizing a polynucleotide comprising a termination polynucleotide sequence to a region of the template which is 5' with respect to hybridization of the composite primer to the template;

(c) extending the composite primer with DNA polymerase;

(d) cleaving the RNA portion of the annealed composite primer with an enzyme that cleaves RNA from an RNA/DNA hybrid such that another composite primer hybridizes to the template and repeats primer extension by strand displacement to produce displaced primer extension product;

(e) hybridizing a polynucleotide comprising a propromoter and a region which hybridizes to the displaced primer extension product under conditions which allow transcription to occur by RNA polymerase, such that RNA transcripts are produced comprising sequences complementary to the displaced primer extension products,

whereby at least 100 copies of RNA transcripts are produced from each displaced primer extension product.

Claim 49 (original): The method of claim 48, wherein between 100 to 1000 copies of RNA transcripts are produced from each displaced primer extension product.